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PTO 2002-4585

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Contractor: _____

Name: MCPriority: 1Sent: 7/30/02Returned: 9-19-02

PTO 02-4585

Japanese Kokai Patent Application
No. Sho 61[1986]-155335

IMMUNOREGULATORS

Masahiro Watanabe et al.

UNITED STATES PATENT AND TRADEMARK OFFICE
WASHINGTON, D.C. SEPTEMBER 2002
TRANSLATED BY THE RALPH MCELROY TRANSLATION COMPANY

JAPANESE PATENT OFFICE (JP)
PATENT JOURNAL (A)
KOKAI PATENT APPLICATION NO. SHO 61[1986]-155335

Int. Cl.⁴: A 61 K 39/395
Sequence No. for Office Use: 8214-4C
Filing No.: Sho 59[1984]-276758
Filing Date: December 28, 1984
Publication Date: July 15, 1986
Number of Inventions: 1 (Total 9 pages)
Examination Request: Not filed

IMMUNOREGULATORS

[Meneki Chosetsuzai]

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Claims

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1. An immunoregulator having at least one kind of active ingredient selected from the blocked Fc fragment of human IgG, the blocked Fab fragment of human IgG, the blocked L chain of human IgG or the blocked H chain of human IgG.

2. The immunoregulator according to the description in Claim 1, of which the form is a liquid preparation for intravenous, muscular or oral administration.

3. The immunoregulator according to the description in Claim 1, of which the form is a powder preparation, tablet preparation, capsule preparation or liposome preparation for oral administration.

* [Numbers in the right margin indicate pagination in the original foreign language text.]

Detailed explanation of the invention

Industrial application field

This invention pertains to immunoregulators having the blocked Fc fragment of human IgG (hereafter, also called blocked Fc fragment), the blocked Fab fragment of human IgG (hereafter, also called blocked Fab fragment), the blocked L chain of human IgG (hereafter, also called blocked L chain) or the blocked H chain of human IgG (hereafter, also called blocked H chain) as the active ingredient.

Prior art

A series of the above blocked substances are obtained by blocking the -SH group of said Fc fragment, Fab fragment, L chain and H chain using appropriate groups.

Among the aforementioned series of blocked substances, those having known pharmacological effects include the alkylated Fc fragment, alkylated Fab fragment, alkylated L chain and alkylated H chain showing anti-ulcer effect in the digestive organs system (Mimura T., Journal of Pharmacobio. Dynamics (Mimura, T. J. Pharm/Dyn.), 6, 397 (1983), but their effects on the immune system of the hosts are not completely known.

Problems to be solved by the invention

The purpose of the present invention lies in providing novel applications for the human IgG-origin substances of blocked Fc fragment, blocked Fab fragment, blocked L chain and blocked H chain.

Means to solve the problems

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In this investigation, the present inventors found that the blocked Fc fragment, blocked Fab fragment, blocked L chain and blocked H chain prepared from human IgG have immune-enhancing or immune-suppressing effects – that is, so-called immunoregulatory effects -- based on the immune response conditions, and thus achieved the present invention. In other words, the present invention pertains to an immunoregulator containing at least one kind of active ingredient selected from the blocked Fc fragment, blocked Fab fragment, blocked L chain and blocked H chain.

It is considered that there is an abnormality in the immune response in collagen disease and rheumatic diseases and that production of autoantibodies by recognizing autosubstances plays an important role in the onset of the diseases. Therefore, it is necessary to correct the abnormal immune reaction in order to treat (the diseases). The targets of immunoregulators are the diseases resulting from immune functional deficiencies of the hosts.

With that having been said, there are known IgG fragments that have been reported, including Fc fragment and Fab fragment of human IgG. For example, there is the report of Porter, et al [Biochem. J., 73, 119 (1959)]. Said Fc fragment or Fab fragment is a polypeptide of 45,000-50,000 molecular weight obtained from degradation of human IgG using papain or plasmin, and the recovery method has been established by the aforementioned Porter, et al.

The blocked Fc fragment and blocked Fab fragment specified as the active ingredients in the present invention can be obtained by splicing the disulfide bonds of the Fc fragment and Fab fragment of IgG, followed by blocking treatment of the -SH group of each resulting fragment.

A typical recovery method for blocked Fc fragment and blocked Fab fragment in the present invention is summarized below.

The pH of a solution containing IgG (protein concentration 2-10%) is adjusted to 6-9, and the solution is treated with papain or plasmin for 10-30 h at 20-40°C. Subsequently, the insoluble [matter] is removed from the treatment solution, and the digested and un-digested products of IgG are separated by the gel filtration method. Ion exchange chromatography (CM-cellulose and DEAE-cellulose) is performed on the digested product, to elute and recover the Fc fragment and Fab fragment of IgG via selective adsorption.

After recovering the Fc fragment and Fab fragment of human IgG, treatment with a reducing agent is carried out to splice the disulfite bonds, followed by blocking treatment (for example, alkylation) to obtain blocked (alkylated) Fc fragment and blocked (alkylated) Fab fragment.

The reducing agents utilized include 2-mercaptoethanol (final concentration 0.75-5.25M), dithiothreitol, and dithioerythritol (final concentration 0.01-0.068M). Blocked Fc fragment or blocked Fab fragment in general is produced by blocking the SH group by a conventional method (Biochemistry, 7, 1950 (1968)). Said blocking reaction is carried out by introducing, for example, the pharmaceutically permitted groups shown below, alkyl group and substituted alkyl groups in particular, by conventional methods. Also, in the detailed explanation, the term "lower" in general means those having 1-4 carbon numbers.

- (1) Lower alkyl groups: methyl, ethyl, n-propyl
- (2) N,N-di-lower alkyl-carbamido-lower alkyl groups: N,N-di-ethyl-carbamidomethyl
- (3) Lower alkoxy carbonyl-lower alkyls: ethoxycarbonylmethyl, ethoxycarbonylethyl
- (4) Carboxy-lower alkyl groups: carboxymethyl, carboxyethyl
- (5) Cyano-lower alkyl groups: cyanomethyl
- (6) β-amino-lower alkyl groups: -CH₂CH₂NH₂, etc.
- (7) Benzoyl-lower alkyl groups: -CH₂COC₆H₅, etc.
- (8) Carbamoyl-lower alkyl groups: -CH₂CONH₂, etc.
- (9) Sulfo group

On the other hand, there are known L chains and H chains of human IgG that have been reported as the IgG-constituting fragments. For example, there was the report by Fleischman, et al [Arch. Biochem. Biophys., Supple. (1), 174 (1962)]. Said L chains and H chains specified as the active ingredients in the present invention are polypeptides of $23,000 \pm 1,000$ and $50,000 \pm 1,500$ molecular weight, respectively, obtained from splicing the disulfite bonds of the IgG from human IgG, and the recovery method has been established by the aforementioned Fleischman, et al. A typical recovery method for L chain and H chain fragments in the present invention is summarized below.

IgG is dissolved at about 2% concentration in 0.55M tris-HCl buffer solution, pH 8.2. Nitrogen gas is passed through the solution slowly, followed by adding 2-mercaptoethanol to a final concentration of 0.75M, to conduct reduction reaction by allowing the solution to stand for 1 h at room temperature. Next, the solution is chilled with an ice-water bath, and the same amount of 0.75M mono-iodoacetamide as the 2-mercaptoethanol are added, and reaction is carried out for 1 h by maintaining the pH of the solution at 8.0 by adding trimethylamine, followed by dialyzing with cold salt water to remove excessive reagent. In this reaction, the free SH group in the L chain or H chain is blocked. Next, dialysis is carried out with 1M propionic acid to release the L chain and H chain, and the solution is passed through a Sephadex G-75 [column] equilibrated with 1M propionic acid to elute the L chain and H chain as 2 peaks. After recovering the L chain and H chain fractions, respectively, conventional treatment methods of dialysis, sterilization-filtration, heating and freezing-drying that can prepare them as pharmaceuticals are conducted.

Of course, not only the substances obtained by the aforementioned methods can be applied as the L chain and H chain of the present invention; L chain and H chain obtained by other applicable methods can also be utilized. Said L chain and H chain are protected by blocking the SH groups, particularly with alkyl and sulfonyl groups that are pharmaceutically permitted. Examples of the substituting groups that are utilized in the blocking reaction are the same as the aforementioned.

Said blocked substances can be produced by conventional methods or by methods derived from them.

Next, experimental examples carried out to verify the pharmacological effects, clinical test, acute toxicity test, dose and dosing method for the blocked Fc fragment, blocked Fab fragment, blocked L chain and blocked H chain of the present invention are shown in the following.

Experimental Example 1 (Effect on production of antibody)

Immunization was conducted on CDF1 mice (7~8-week-old, male) by intravenous administration of 2×10^6 sheep red blood cells (SRBC). After 4 days, the spleens were extracted and the number of antibody-producing cells (hemolytic plaque forming cell) was determined to investigate the effect of the test drug on antibody production. The determination of the number of the antibody-producing cells was performed based on the method of Cunningham, et al [Immunology, 14, 599 (1968)]. Here, the administrations of carbamoylmethylated Fc fragment, carbamoylmethylated Fab fragment, carbamoylmethylated L chain and carbamoylmethylated H chain as the test drugs were carried out by intravenous administration immediately after SRBC was administered as the antigen. Also, each group consisted of 4 animals. The results are shown in Table 1.

Compared to the number of anti-SRBC antibody-producing cells in the spleens of the control group, significant promotion of antibody production was observed in the cases where carbamoylmethylated Fc fragment, carbamoylmethylated Fab fragment, carbamoylmethylated L chain and carbamoylmethylated H chain were administered.

Experimental Example 2 (Effect on host with reduced immune functions)

Immune suppressing agents were administered to CDF1 mice (7~8-week-old, male) based on the following condition, to create a condition of immune function deficiency. Azathioprine (40 mg/kg), cyclophosphamide (50 mg/kg), or betamethasone (1 mg/kg) was administered 4 days, 3 days and 2 days prior to antigen stimulation. The administration pathway of these drugs was intra-peritoneal injection. Subsequently, sheep red blood cell (SRBC) (2×10^6) as the antigenic stimulator as well as carbamoylmethylated Fc fragment, carbamoylmethylated Fab fragment, carbamoylmethylated L chain or carbamoylmethylated H chain as the test drug were administered at the same time, and the number of antibody-producing cells in the spleens after 4 days were determined and the effects on the hosts with reduced immune functions were investigated. In this experiment, levamisole, which is an immune regulating/treatment drug, was selected as the control drug. Levamisole was administered by intra-peritoneal injection while all other drugs were by intravenous injection. Also, each group consisted of 4 animals. The results of the above (experiment) are shown in Table 2.

Compared to the non-treatment group, the group administered with the immune suppresser showed a decreasing number of antibody-producing cells. On the other hand, even in the hosts with such reduced immune functions, the numbers of antibody-producing cells were found to have increased in the groups administered with carbamoylmethylated Fc fragment, carbamoylmethylated Fab fragment, carbamoylmethylated L chain and carbamoylmethylated H chain.

Experimental Example 3 (Effect on hosts with elevated immune function)

It was reported that, when colchicine was administered together with an antigen, antibody production was elevated because the derivation of suppresser T cells was hindered (J. Exp. Med., 147, 1213 (1977)). In this experiment, TNP was introduced as hapten to keyhole limpet hemocyanin (KLH), based the method of Haba, et al. (Meneki Jikken Sosa Ho (Immunology Experimental Methods), p. 1129 (1971), Nippon Meneki Gakkai (J. of Japanese Association of Immunology)). In other words, using TNP-KLH as the antigen. TNP-KLH (200 µg/mouse) and bentonite as the adjuvant were administered to CDF1 mice by intra-peritoneal injection, and in the meantime, colchicine (1 mg/kg) was administered by intra-peritoneal injection as well. Furthermore, carbamoylmethylated Fc fragment, carbamoylmethylated Fab fragment, carbamoylmethylated L chain or carbamoylmethylated H chain as the test drug was administered by intravenous injection, and the number of anti-TNP antibody-producing cells in the spleens after 6 days was determined and the effects on the hosts with elevated immune functions were investigated. Here, for the experimental groups, each group consisted of 4 animals. The results are shown in Table 3.

Compared to the non-treatment group, the control group treated with colchicine showed an obvious increase in the number of antibody-producing cells, revealing a condition that is considered as a so-called exacerbated immune function. On the other hand, the immune responses of the groups administered with carbamoylmethylated Fc fragment, carbamoylmethylated Fab fragment, carbamoylmethylated L chain and carbamoylmethylated H chain showed values close to that of the non-treatment group (normal mice), revealing a tendency toward suppression of the elevated immune functions of the hosts.

Experimental Example 4 (Effect on adjuvant arthritis as a rheumatic model)

Mycobacterium butyricus (Disk) suspended in liquid paraffin at 0.5 mg/50 µL was administered by subcutaneous injection to the soles of the feet of Wistar male rats of about 200 g body weight under ether anesthesia. Carbamoylmethylated Fc fragment, carbamoylmethylated Fab fragment, carbamoylmethylated L chain or carbamoylmethylated H chain as the test drug was administered by intravenous injection for 20 days at 20 mg/kg/d from the day of adjuvant treatment. Also, D-penicillamine, which is a rheumatic treatment drug, was administered as a control drug by intra-peritoneal injection. Afterward, the volumes of the two feet were determined using a device for measuring feet swelling volume. Also, one group consisted of 7 animals in this experiment. The results are shown in Table 4.

The changes of swelling rates of the feet after adjuvant treatment were shown for day 10 and day 20. However, with respect to the delayed swelling from day 7 and on, the groups

administered with carbamoylmethylated Fc fragment, carbamoylmethylated Fab fragment, carbamoylmethylated L chain and carbamoylmethylated H chain showed apparent suppressing effects on adjuvant arthritis, compared to the control group.

Dose and dosing methods

From the results of the above experiments, the blocked Fc fragment, blocked Fab fragment, blocked L chain and blocked H chain are preferred to be administered at 1-100 mg/kg per day for adults.

The drugs of the present invention can be administered as injection preparations or as oral preparations. When administered as an injection drug, for example, the drugs can be dissolved in distilled water for injection purposes. The dosing method in general is intravenous injection or muscular injection. When utilized as oral agents, they can be administered as capsules, tablets, powders or liposome preparations or as liquid oral preparations. For oral preparations, an enteric-soluble form may be used. These drug forms can be produced by conventional methods known to those skilled in the art, for example, the methods listed in the Japan Pharmacopoeia.

Effect/efficacy

The blocked Fc fragment, blocked Fab fragment, blocked L chain and blocked H chain pertaining to the present invention have extremely low toxicity with significantly potent immunoregulatory effect, and are therefore considered extremely useful as treatment and preventative drugs (that is, immunoregulators) for diseases originating from immune deficiencies including rheumatic diseases and collagen diseases. Also, from the effect against adjuvant arthritis, for example, the concept of immunoregulator in the present invention includes anti-inflammatory agents.

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Reference examples/application examples

Reference examples with respect to the production methods of the blocked Fc fragment and Fab fragment, as well as applications examples of the present invention are shown in the following.

Reference Example 1

Sixty milligrams of sodium azide was added to 3% IgG solution (60 ml), and the pH was adjusted to 7.5 with 1N NaOH solution. Plasmin was added to a final concentration of 4 cu/mL and digestion treatment was carried out for about 15 h at 35°C. The pH was corrected to 6.5 after the treatment, and after standing for 1 h at 4°C, the insoluble was removed by centrifugation. The solution digested with plasmin (about 60 ml) was injected onto a Sephadex G-200 column to

carry out gel filtration to separate the undigested globulin (7s) and the digested products (Fab+Fc). Said digested products were then put on a CM-cellulose column (pH 7.0) to have the Fab fragment and Fc fragment adsorbed. Washing was carried out with the column, followed by development with a 0.01M phosphate buffer solution (pH 7.0) added with 0.3M NaCl, to recover the Fab and Fc fragments.

The 2 obtained fragments were dissolved in 0.05M tris-HCl buffer solution (pH 8.2) at about 2% concentration, and 2-mercaptoethanol was added to a final concentration of 0.75-5.25M, to splice the disulfite bonds. Subsequently, 0.75-5.25M iodoacetic acid was added, and after reacting for 1 h while maintaining the pH at 8.0, the excessive sample was removed with a Sephadex G-25 column. Next, dialysis was carried out with physiological saline solution, followed by sterilization/filtration, to obtain a freeze-dried product.

Reference Example 2

Five milligrams of papain was added to 2.5% IgG solution (20 ml; 0.02M EDTA-0.05M phosphate buffer solution, pH 7.5) and digestion treatment was carried out for about 10-20 min at 37°C, followed by cooling with ice water. Fractionation with a Sephadex G-150 column was carried out to obtain the 7s fraction, which was treated with dithiothreitol at a final concentration of 0.01M for 2 h at room temperature and at pH 7.5-8.0. Subsequently, iodoacetamide was added at final concentration of 0.2M to carry out a reaction for 1 h while cooling with ice. Dialysis was conducted with 0.005M tris-HCl, pH 8.0, and the resulting crystals were separated by centrifugation. The supernatant was crude blocked Fab fragment, and Fc fragment adsorbed was recovered as the precipitating fraction.

Reference Example 3

IgG was dissolved in 0.05M tris-HCl buffer solution (pH 8.2) at about 2% concentration, and 2-mercaptoethanol was added to a final concentration of 0.75M, to splice the disulfite bonds. Next, 0.7M iodoacetic acid was added to carry out reaction for 1 h while maintaining the pH at 8.0, and the excessive reagent was removed by a Sephadex 25 column. Subsequently, the solution was applied to a Sephadex G-200 column (4.0x120 cm) in the presence of SDS (sodium dodecyl sulfate) (solvent: 0.04M SDS-0.05M phosphate buffer solution (pH 8.0)) to carry out measurement at O.D. 280 nm and recover L chain and H chain fractions. Next, SDS was removed from the L chain fraction or from the H chain fraction, and dialysis was carried out with physiological saline solution, followed by sterilization/filtration, to obtain a freeze-dried product.

Application examples of the present invention are shown in the following.

Application Example 1 (Oral preparation)

(1) Carbamoylmethylated Fc fragment, carbamoylmethylated Fab fragment, carbamoylmethylated L chain or carbamoylmethylated H chain from human IgG	5.0 mg
(2) Micro particle No. 209 for direct pressing (product of Fuji Kagaku K.K.)	46.6 mg
Magnesium metasilicate-aluminate	20%
Corn starch	30%
Lactose	50%
(3) Crystalline cellulose	24.0 mg
(4) Calcium carboxymethylcellulose	4.0 mg
(5) Magnesium stearate	0.4 mg

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Pre-sieving of (1), (3) and (4) with an 100-mesh sieve was carried out, respectively. The obtained (1), (3) and (4) and (2) were dried, respectively, to a given water content, followed by mixing at the aforementioned blending weights using a mixer. After the mixed powder became homogeneous, (5) was added and mixed for a short time (30 sec) and the mixed powder was subjected to tablet pressing (Rod: 6.3 mmφ, 6.0 mmR), and tablets of 80 mg/tablet were prepared.

The tablets may be coated with a gastric-soluble film coating agent (for example, polyvinylacetal diethylaminoacetate) or (blended with) edible coloring agent if necessary.

Application Example 2 (Intravenous injection preparation)

(1) Carbamoylmethylated Fc fragment, carbamoylmethylated Fab fragment, carbamoylmethylated L chain or carbamoylmethylated H chain from human IgG	50 mg
(2) Glucose	100 mg
(3) Physiological saline solution	10 mL

(1) and (2) were added to (3) according to the aforementioned blending ratio and agitated until total dissolution. After the solution was filtered with a membrane filter of 0.45 μ opening, sterilization-filtration was carried out using a membrane filter of 0.20 μ opening. The filtered solution was filled into 10 mL vials under sterilized condition, and after nitrogen gas was filled, the vials were sealed to prepare intravenous injection preparation.

Application Example 3 (Capsules preparation)

(1) Carbamoylmethylated Fc fragment, carbamoylmethylated Fab fragment, carbamoylmethylated L chain or carbamoylmethylated H chain from human IgG	50 g
(2) Lactose	935 g
(3) Magnesium stearate	15 g

The above ingredients were weighed, respectively, to a total of 1000 g, and mixed homogeneously. The mixed powder was then filled in hard gelatin capsules at 200 mg each capsule.

Application Example 4 (Liposome preparation)

Carbamoylmethylated Fc fragment, carbamoylmethylated Fab fragment, carbamoylmethylated L chain or carbamoylmethylated H chain from human IgG was dissolved at about 5% concentration in 0.01M phosphate buffer solution (pH 7.2) containing 0.125M NaCl.

Separately, 100 mg of egg yolk phospholipid containing 0.5, 10 and 20% (w/w) each of phosphatidic acid was dissolved in 10 mL chloroform, and phospholipid film was formed using a rotary evaporator. One mL of a solution of the aforementioned carbamoylmethylated Fc fragment, carbamoylmethylated Fab fragment, carbamoylmethylated L chain or carbamoylmethylated H chain was then added and enclosed fatty droplets were formed by shaking, to give a liposome preparation in which the drugs were embodied.

Table 1

① 試験	② 投与量 (mg/kg)	抗SRBC ③ 抗体産生細胞数/脾臓 ($\times 10^4$)	④ 促進率%
⑤ 対照 (生理食塩水) 天然 IgG	- 25	10.2 ± 2.5 10.9 ± 2.7	(108) (107)
⑥ カルバモイルメチル化Fc断片 ⑦ Fc断片	25 ~ 7.5 2.5 25 7.5	48.2 ± 3.2 (47.7 ± 10.2) 36.5 ± 10.9 11.2 ± 3.7 11.4 ± 1.7	(473) (399) (358) (109) (111)
カルバモイルメチル化Fab断片 ⑨ Fab断片	25 7.5 2.5 25 7.5	20.1 ± 9.1 19.0 ± 3.8 18.1 ± 5.4 10.5 ± 1.3 9.9 ± 0.8	(197) (184) (158) (103) (96)
カルバモイルメチル化L链 ⑩ L链	25 7.5 2.5 25 7.5	43.1 ± 12.8 (40.9 ± 9.5) 33.8 ± 6.2 18.4 ± 3.7 18.8 ± 2.4	(423) (401) (324) (102) (108)
カルバモイルメチル化H链 ⑫ H链	25 7.5 2.5 25 7.5	22.7 ± 7.3 17.3 ± 8.9 15.6 ± 7.3 11.4 ± 3.0 10.7 ± 1.5	(223) (189) (153) (112) (105)
(13) 平均 ± 標準偏差			

- Key 1 Treatment
 2 Dose
 3 Number of anti-SRBC antibody-producing cells/spleen
 4 Rate of promotion
 5 Control (physiological saline solution)
 6 Natural

- 7 Carbamoylmethylated Fc fragment
 8 Fragment
 9 Carbamoylmethylated Fab fragment
 10 Carbamoylmethylated L chain
 11 Chain
 12 Carbamoylmethylated H chain
 13 Mean \pm standard deviation

Table 2

① 免疫抑制 下処理	② 投与薬剤 (25mg/kg)	抗SRBC 抗体産生細胞数/脾臓 ($\times 10^3$)	無処置マウスに対する 抗体産生細胞数の割合 (%)
無処置 ⑥ 対照 (生理食塩水) ⑤ ⑦	カルバモイルメチル化Fc断片 Fab断片 L链 H链 レバミゾール⑩ ⑨	163.8 \pm 33.7 345.5 \pm 64.9 291.5 \pm 20.3 328.4 \pm 45.1 305.9 \pm 21.6 351.6 \pm 31.7	(100) (211) (178) (201) (187) (215)
⑪ アザチオリノン ナフチドロ 処理 ⑫	カルバモイルメチル化Fc断片 Fab断片 L链 H链 Fc断片 Fab断片 L链 H链 レバミゾール⑩ ⑨	15.7 \pm 3.4 17.3 \pm 2.1 70.8 \pm 16.6 34.7 \pm 7.4 75.6 \pm 10.9 38.6 \pm 8.2 17.8 \pm 1.4 15.5 \pm 0.9 18.5 \pm 1.7 14.9 \pm 0.8 37.8 \pm 11.8	(9.6) (18.8) (43.2) (21.2) (46.2) (18.7) (10.8) (9.5) (10.1) (9.0) (22.9)
シクロホスカ アツミン 処理 ⑬	対照 (生理食塩水) 天然Fc カルバモイルメチル化Fc断片 Fab断片 L链 H链 Fc断片 Fab断片 L链 H链 レバミゾール⑩ ⑨	16.8 \pm 6.7 16.2 \pm 6.8 26.0 \pm 6.6 26.7 \pm 4.3 27.1 \pm 5.4 19.3 \pm 3.6 18.5 \pm 4.2 13.9 \pm 3.6 13.5 \pm 9.3 16.1 \pm 4.8 21.6 \pm 6.8	(10.2) (9.9) (15.9) (12.6) (16.5) (11.8) (10.1) (9.7) (9.5) (10.6) (13.8)
ベタメタ ゾン処理 ⑭	対照 (生理食塩水) 天然Fc カルバモイルメチル化Fc断片 Fab断片 L链 H链 Fc断片 Fab断片 L链 H链 レバミゾール⑩ ⑨	62.8 \pm 21.1 59.6 \pm 17.3 104.3 \pm 31.2 98.2 \pm 27.1 118.5 \pm 25.3 87.8 \pm 18.2 58.6 \pm 17.3 60.7 \pm 20.8 61.2 \pm 14.9 57.3 \pm 25.4 41.6 \pm 15.4	(38.3) (36.4) (63.7) (68.6) (78.3) (53.7) (35.8) (37.1) (37.4) (34.9) (25.4)

(15) 平均土標準偏差

- Key 1 Treatment for reducing immune functions
 2 Administered drug
 3 Number of anti-SRBC antibody-producing cells/spleen
 4 Ratio to the number of antibody-producing cells with respect to non-treatment mice
 5 Non-treatment
 6 Control (physiological saline solution)
 7 Carbamoylmethylated Fc fragment
 8 Fragment
 9 Chain
 10 Levamisole
 11 Treatment with azathioprin [sic; azathioprine]
 12 Natural
 13 Treatment with cyclophosphamide
 14 Treatment with betamethasone

15 Mean \pm standard deviation

Table 3

① 免疫機能亢進処置	② 投与薬剤 (25mg/kg)	③ 汗TNP抗体産生細胞数/脾臓 ($\times 10^2$)	④ 感染マウスに対する抗体産生細胞数の割合 (%)
⑤ 無処置	-	83.0 \pm 22.7	(100)
コルヒチン チン 処置	対照 (生理食塩水) ⑦ 天冬酰胺 ⑧ カルバモイルメチル化Fc断片 ⑨ Fab断片 ⑩ L链 ⑪ H链 ⑫	136.7 \pm 34.4 128.1 \pm 78.0 82.0 \pm 23.2 108.4 \pm 28.4 93.7 \pm 18.8 114.0 \pm 31.2 140.8 \pm 25.8 139.1 \pm 14.4 138.4 \pm 2.9 151.7 \pm 30.6	(217) (203) (190) (172) (149) (181) (223) (221) (219) (241)
		(12) 平均 \pm 標準偏差	

- Key 1 Treatment for stimulating immune functions
 2 Administered drug
 3 Number of anti-TNP antibody-producing cells/spleen
 4 Ratio to the number of antibody-producing cells with respect to non-treatment mice
 5 Non-treatment
 6 Colchicine treatment
 7 Control (physiological saline solution)
 8 Natural
 9 Carbamoylmethylated Fc fragment
 10 Fragment
 11 Chain
 12 Mean \pm standard deviation

Table 4

① 投与薬剤 (20mg/kg/日) ②	③ 肿脹率 (%) ■	
	10日目 ④	20日目 ⑤
⑥ 対照 (生理食塩水) ⑦	92.5 \pm 38.4	215.3 \pm 85.6
カルバモイルメチル化Fc断片 ⑨	38.9 \pm 29.7	94.7 \pm 31.3
Fab断片 ⑩	54.7 \pm 11.9	132.2 \pm 50.0
L链 ⑪	40.4 \pm 17.4	98.1 \pm 26.8
H链 ⑫	49.2 \pm 30.6	153.2 \pm 37.3
Fc断片 ⑨	38.1 \pm 20.5	158.5 \pm 64.1
Fab断片 ⑩	33.4 \pm 27.9	224.0 \pm 93.7
L链 ⑪	30.7 \pm 15.3	206.4 \pm 42.8
H链 ⑫	32.8 \pm 31.6	213.7 \pm 69.3
ローベニシラミン ⑭	60.4 \pm 18.0	118.6 \pm 41.2

(11) ■ アジュvant処置後の浮腫容積 - アジュvant処置前の浮腫容積 $\times 100$
 (12) ■ 基本平均 \pm 標準偏差

- Key 1 Drug administered
 2 Day
 3 Swelling rate
 4 Day 10
 5 Day 20

- 6 Control (physiological saline solution)
- 7 Carbamoylmethylated Fc fragment
- 8 Fragment
- 9 Chain
- 10 D-penicillamine
- 11 Swelling rate after Adjuvant treatment - Swelling rate before Adjuvant treatment
- 12 Swelling rate before Adjuvant treatment
- 13 Mean ± standard deviation